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Hepatic Portal Vein Cannulation for Infusion and Blood Sampling in Freely Moving Rats

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STRUBBE, J. H., J. E. BRUGGINK AND A. B. STEFFENS. *Hepatic portal vein cannulation for infusion and blood sampling in freely moving rats.* *PHYSIOL BEHAV* **65**(4/5) 885–887, 1999.—Chronic portal vein cannulation in the rat is an important technique to study secretory rates of hormones from the endocrine pancreas. Moreover, it can be used for studying the effects of enteric hormones and pharmacological agents on behavioral and physiological processes. This article contains an extensive description of a cannulation technique of the portal vein that has many advantages over those reported so far in the literature, and that was very successful in several behavioral and physiological studies during the last decade. © 1999 Elsevier Science Inc.

Hepatic portal vein Rat Blood sampling Portal infusion

FROM the intestines absorbed fuels, ingested pharmacological agents, and enteric hormones are transported via the mesenteric veins to the main stream of the hepatic portal vein. Also, the hormones of the pancreas are released into the portal vein. Many portal blood compounds (fuels, metabolites, hormones, pharmacological agents) are acting, modified, or extracted in the liver (1,3,4,13,14). Therefore, for several studies, changes in portal vein of fuels and hormones may give a better and more direct assessment of absorption and secretory rates than changes in the peripheral circulation (1,3,6,12). In combination with flow measurements the absorption and secretory rates can be measured even more exactly. Because the liver is the first organ that receives absorbed fuels and intestinal and pancreatic hormones, it plays an important role in energy metabolism and the regulation of food intake (1,3,7).

For all these studies a chronic catheter in the portal vein is necessary. Several techniques for catheterization have been developed in the past. In humans, the catheterization of the umbilical vein has been performed (2). In dogs cannulations of mesenteric and pancreatic veins have been described (9). However, for small animals such as the rat, more refined techniques are needed. In 1977 we described a technique by which a cannula with a very small diameter (0.64 mm) was inserted via a side branch of the portal vein. The tip was pushed retro-

grade until the tip of this cannula ended in the main stream of the portal vein (12). A similar technique was described by others a few years later (5). Because in these methods permanent clamping of veins occurred with possible necrosis of the downstream tissue, we developed a method in which a cannula with an outer diameter of 1.0 mm was inserted freely in the widest part of the portal vein and fixed with a suture around the vein (13). This fixation method occasionally caused disturbances of blood flow and unexpected bleeding, so that we improved this method. In this article a cannulation technique of the portal vein is described, which was very successful during the last decade in several behavioral and physiological studies (1,3,6,13). Moreover, this technique has several advantages over other techniques described in the past.

METHOD

The Preparation of the Cannula

A silastic medical-grade tubing (i.d. 0.5 mm, o.d. 1.0 mm, Maxxim Medical Europe B.V., 's-Hertogenbosch, The Netherlands) of 22 cm is beveled at one end to a point. At a distance of 1 mm from the tip a hole was cut with a sharp cut 20 G needle. A very small ring of silicon glue (Rhone-Poulenc, Saint-Fons, France) is placed at 1 cm, and a somewhat larger at 5 cm

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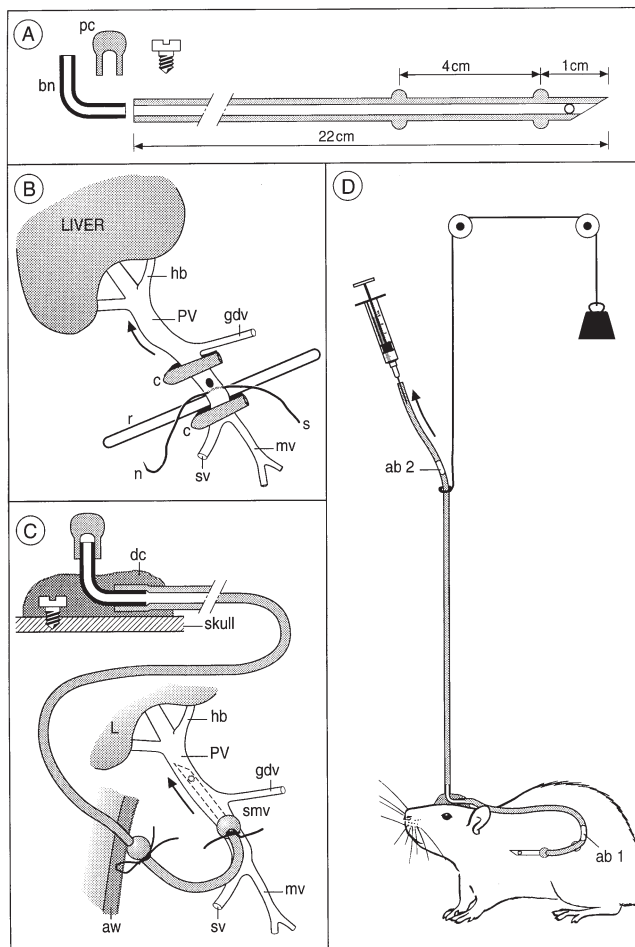


FIG. 1. Schematic representation of: (A) materials: silicon cannula, screws, stainless bent needle (bn) polyethylene cap (pc). (B) The area of surgery: liver (L), portal vein (pv), hepatic branches of the portal vein (hb), gastroduodenal vein (gdv), superior mesenteric vein (smv), splenic vein (sv), and mesenteric veins (mv). Needle (n), clamps (c), rod (r), and suture (s) are indicated. Arrow indicates the flow direction. The hole in the vein is indicated as a black spot. (C) Implanted cannula in the portal vein and connection with the abdominal wall (aw) and fixation on the skull with dental cement (dc). (D) Rat in cage connected with tubings for blood sampling by a syringe. Air bubbles (ab) 1 and 2 are indicated in the tubing.

from the cannula tip (see Fig. 1A). Before surgery cannulas are sterilized by autoclaving.

Surgery

The sterilized cannula is filled with heparin solution in saline (100 IU/mL). Under anesthesia the ventral abdominal wall is exposed. The hairs at the place of incision, i.e., 2–4 cm caudal to the xiphoid process, are removed. The skin is sterilized with a chlorhexidine solution. To expose the liver and duodenal loop, a midline incision of about 5 cm is made in the skin and in the abdominal muscular wall. The duodenal loop is turned to the right, so that the portal vein is exposed. Using blunt bent forceps the venous part of the superior mesenteric vein is separated from the surrounding tissue between the en-

trance of the gastroduodenal and splenic vein, and briefly clamped by use of two smoothly closing small clamps, leaving a compartment of 0.5 cm free of blood pressure. It is important that the small clamps have no sharp edges and do not give too much pressure on the vein. Heifetz clips are very suitable for this purpose (Edward Weck & Co., Inc. Durham, NC, Cat. No. 659115). A horizontally placed very smooth rod (o.d. 1–2 mm) placed under the vein may be helpful for the manipulation of clamps and other surgical tools.

One stitch is made through the superficial part of the venous wall of the distal end of the clamped compartment with a BV1 needle connected to a piece of silk thread (7-0) (Ethycon Perma Hand Seide, Art no. K804) (Fig. 1B). The suture ends are tied together to a knot. Care should be taken, that these ends are not tied too tight to the venous wall because this will then cause too much tension on the vein. It is important that clamping occurs as short as possible (preferable less than 3 min) to prevent hemorrhages in the upstream area. To prevent damaging of the hindwall of the compartment during puncture, the upstream clamp is released for a while to fill the compartment with blood. Then using the tip of a 20-G needle a hole is made with care in this venous compartment about 1 mm downstream from the stitch. Immediately thereafter the cannula is inserted, the downstream clamp is removed, and subsequently the cannula is pushed downstream up to the silicon ring. The loose ends of the stitch and knot are now tied to a knot over the cannula just behind the silicon ring (Fig. 1C). The upstream clamp is removed now and the duodenal loop is turned back to its original position.

The second ring in the cannula is sutured onto the abdominal wall. This ring prevents tension on the just implanted cannula. The cannula is inserted under the skin and tunneled to a midline incision on the crown of the head by means of a hemostatic forceps, where it is connected to a stainless steel L-shaped bent needle (20 G). The cannula is then filled with 0.15–0.2 mL, 55% (W/V) polyvinylpyrrolidone (PVP) (MW 25,000, Merck, Cat. No. 107443) in heparin (500 IU/mL saline) and closed with a polyethylene cap. The heparin for the PVP contains a bacteriostatic agent. The abdominal muscle and skin walls are closed now.

Four small holes are drilled in the parietal and frontal bones of the skull and screws (diameter 1.6 mm) are placed in these holes. Subsequently, the cannula and bent needle are directly anchored onto the skull with dental acrylic around the screws, and the skin is sutured (Fig. 1C). This anchoring procedure is different from the method described previously, where we used the L-shaped bent needle in a piece of perspex (10). The first day after surgery, the cannula should be checked and refilled with the PVP solution. Rats were allowed to recover from surgery for at least 1 week before they were used in the experiments. The cannula was checked twice a week to prevent blockade inside the cannula by blood clotting. Cannulas remained patent for withdrawal of blood samples in about 90% of our rats for up to 1 month and up to 80% after 2 months. However, even when no blood could be withdrawn, infusions were still possible up to at least 2 months after surgery. These percentages are dependent on the experience of the surgeon.

Sampling and Infusion Procedure

The procedure is the same as described previously (10). In short, when an experiment is started, a long polyethylene tubing (i.d. 0.75 mm, o.d. 1.45 mm), which is filled with saline 0.9% NaCl, is attached to the bent needle on top of the skull.

The tubing is tautened by a counterweight, and the end is just outside the cage. It can now be used for blood sampling and/or intraportal infusion. In case of long-term overnight infusion a small swivel joint (11) should be used preventing torsion of the tubing. In rats weighing 350 g, volumes of 0.1 mL/min can be used for short-term experiments (10–20 min), whereas in long term (1–24 h) infusions smaller volumes are preferred. A stainless steel spring around the tubing will protect it against biting. A 1-mL syringe with a 20-G is inserted into the tubing outside the cage and the PVP solution is withdrawn from the cannula. Blood is coming up in the cannula now. It is very important that blood clotting is avoided. This can be prevented by relatively rapid sampling in combination with some anticoagulant (heparin or citrate). It is very important that the sampling procedure will be followed as described earlier for the chronic jugular catheter (10). However one should keep in mind that the portal cannula is much longer than the jugular catheter. This means that the first air bubble [see (10) and Fig. 1D], which separates blood from saline in the tubing, is pushed with a syringe filled with saline solution over a longer distance than when blood is sampled from a jugular catheter. The exact distances should be measured by means of a dummy probe. The marker air bubble at the syringe end will tell the investigator where the first air bubble is situated in the cannula inside the animal with a small compartment of anticoagulant just before the portal blood (Fig. 1D). This sampling procedure including the use of air bubbles is absolutely necessary to avoid effects of dead volumes and blood clotting. Because blood flow and pressure are relatively high in the portal vein, rapid sampling of volumes ranging from 0.1–0.4 and with intervals of 1–2 min is possible (3,6). Stress on the rat due to loss of blood during repeated sampling should be avoided by transfusing blood from donor rats at regular intervals.

DISCUSSION

In this article a cannulation technique of the portal vein is described, which was very successful during the last decade in several behavioral and physiological studies (1,3,6,13). The present technique has at least three main advantages over some techniques described in the past: 1) disturbances to intestinal blood flow are minimal, because only a very small part of the cannula is inserted freely in the widest part of the portal vein. No veins are clamped permanently as was the case in other techniques (5,12). Of course, it is also possible to use cannulas with a smaller diameter as described previously (12). However, they allow less rapid blood sampling and they are more fragile than the 1-mm cannulas. 2) Cannula longevity is enhanced such that cannulas can be used for 1–2 months for blood sampling and for at least 2 months for infusion. 3) No special techniques as used in microsurgery are required (8). Only one stitch is needed to fix the cannula onto the vein. 4) Rapid blood sampling with short intervals (1–2 min.) is possible, without disturbing the animal. The relative high blood flow through the portal bed allows this rapid sampling procedure. It is also possible to use this portal sampling or infusion in combination with simultaneous blood sampling from the right atrium (3,6) via a chronically implanted jugular catheter (10).

Although some skills need to be developed, experimenters who are already performing cannulation surgery on rats or other small animals, should be able to perform this technique, which has a wide application in the field of physiology and behavior.

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